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The molecular basis of TnrA control by glutamine synthetase in bacillus subtilis

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Abstract

© 2016 by The American Society for Biochemistry and Molecular Biology, Inc.TnrA is amaster regulator of nitrogen assimilation in Bacillus subtilis. This study focuses on the mechanism of how glutamine synthetase (GS) inhibits TnrA function in response to key metabolites ATP, AMP, glutamine, and glutamate. We suggest a model of two mutually exclusive GS conformations governing the interaction with TnrA. Inthe ATP-bound state (A-state), GS is catalytically active but unable to interact with TnrA. This conformation was stabilized by phosphorylated L-methionine sulfoximine (MSX), fixing the enzymein the transition state. When occupied by glutamine (or its analogue MSX), GS resides in a conformation that has high affinity for TnrA (Q-state). The A-and Q-state are mutually exclusive, and in agreement, ATP and glutamine bind to GS in a competitive manner. At elevated concentrations of glutamine, ATP is no longer able to bind GS and to bring it into the A-state. AMP efficiently competes with ATP and prevents formation of the A-state, thereby favoring GS-TnrA interaction. Surface plasmon resonance analysis shows that TnrA bound to a positively regulated promoter fragment binds GS in the Q-state, whereas it rapidly dissociates from a negatively regulated promoter fragment. These data imply that GS controls TnrA activity at positively controlled promoters by shielding the transcription factor in the DNA-bound state. Accordingtosize exclusion and multiangle light scattering analysis, the dodecameric GS can bind three TnrA dimers. The highly interdependent ligand binding properties of GS reveal this enzyme as a sophisticated sensor of the nitrogen and energy state of the cell to control the activity of DNA-bound TnrA.

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